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Exercise alone or in combination with environmental heat stress can elevate blood S-100B protein concentrations. However, the explanatory power of exercise with marked environmental heat stress on the appearance of S-100B is questionable. It is possible that the process of heat acclimation might afford additional insight. Purpose: Determine the S-100B response to moderate intensity exercise with heat strain before and after heat acclimation. Methods: Nine healthy male volunteers completed 10 consecutive days of heat acclimation consisting of up to 100 minutes of treadmill walking (1.56m/s, 4% grade) in the heat (45°C, 20% relative humidity). Changes in heart rate (HR), rectal temperature (Tre), and sweat rate (SR) were examined to determine successful acclimation. Area under the curve (AUC) for Tre > 38.5°C was calculated to assess cumulative hyperthermia. Blood samples were taken before and after exercise on days 1 and 10 and analyzed for serum osmolality and S-100B concentration. Results: All subjects displayed physiological adaptations to heat acclimation, including a significant ($P < 0.05$) reduction in final HR (161 to 145 b/min) and Tre (39.0 to 38.4° C), as well as a modest (~10%) increase in SR (1.10 to 1.20 L/hr; $p = 0.09$). No differences were observed in pre-to-post exercise serum S-100B concentrations on day 1 or day 10, nor were differences observed in S-100B values between days 1 and 10. No significant correlations were found between S100B values and any variable of interest. Conclusions: S-100B concentrations do not necessarily increase in response to exercise heat strain and no effect of heat acclimation on S-100B could be observed despite other quantifiable physiological adaptations.

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Serum S-100β Response to Exercise–Heat Strain before and after Acclimation

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ABSTRACT

CHEUVRONT, S. N., T. D. CHINEVERE, B. R. ELY, R. W. KENEFICK, D. A. GOODMAN, J. P. MCCLUNG, and M. N. SAWKA. Serum S-100β Response to Exercise–Heat Strain before and after Acclimation. *Med. Sci. Sports Exerc.*, Vol. 40, No. 8, pp. 1477–1482, 2008. Exercise alone or in combination with environmental heat stress can elevate blood S-100β protein concentrations. However, the explanatory power of exercise with marked environmental heat stress on the appearance of S-100β is questionable. It is possible that the process of heat acclimation might afford additional insight. **Purpose:** Determine the S-100β response to moderate-intensity exercise with heat strain before and after heat acclimation. **Methods:** Nine healthy male volunteers completed 10 consecutive days of heat acclimation consisting of up to 100 min of treadmill walking (1.56 $m \cdot s^{-1}$, 4% grade) in the heat (45°C, 20% relative humidity). Changes in HR, rectal temperature (T_{re}), and sweat rate (SR) were examined to determine successful acclimation. Area under the curve (AUC) for T_{re} greater than 38.5°C was calculated to assess cumulative hyperthermia. Blood samples were taken before and after exercise on days 1 and 10 and were analyzed for serum osmolality and S-100β concentration. **Results:** All subjects displayed physiological adaptations to heat acclimation including a significant ($P < 0.05$) reduction in final HR (161 to 145 bpm) and T_{re} (39.0 to 38.4°C), as well as a modest (~10%) increase in SR (1.10 to 1.20 $L \cdot h^{-1}$; $P = 0.09$). No differences were observed in pre- to postexercise serum S-100β concentrations on day 1 or 10, and no differences were observed in S-100β values between days 1 and 10. No significant correlations were found between S-100β values and any variable of interest. **Conclusions:** S-100β concentrations do not necessarily increase in response to exercise–heat strain, and no effect of heat acclimation on S-100β could be observed despite other quantifiable physiological adaptations.
Key Words: BLOOD–BRAIN BARRIER, THERMOREGULATION, HEAT ACCLIMATIZATION, HYPERTHERMIA

The glial protein S-100β exists in the blood of normal healthy humans at low concentrations (~0.03 $\mu g \cdot L^{-1}$) (1). For this reason, small increases (~0.10 to 0.30 $\mu g \cdot L^{-1}$) may be demonstrable of increased blood–brain barrier (BBB) permeability (14,17) and larger increases (>1.0 $\mu g \cdot L^{-1}$) of traumatic brain injury (10,14,19). It is also clear, however, that S-100β is expressed in skeletal muscle and other tissues (8), can increase dramatically in response to extracranial tissue damage (1,10), and is more modestly elevated in response to acute exercise (5,9,19,24,30,32,33). It appears that S-100β increases proportionally with increasing exercise intensity and duration (9,24), is influenced by exercise mode (5,24), and can stay elevated postexercise for several hours (9,19). Elevated S-100β levels may simply be a reflection of muscle damage resulting from exhaustive high-intensity exercise (9), but it is also possible that differences in body heat storage that occur with heat strain may play a role.

Severe hyperthermia is associated with an increase in the permeability of the BBB in animals (12,28,29). This could result in the free exchange of metabolic substances between the brain and the periphery—some of which are implicated in fatigue (28). Although modest elevations in core body temperature (+1.5°C) resulting from water immersion produce no change in serum S-100β in humans (31), slightly larger (+2.0–2.5°C) increases resulting from the combination of intense exercise and environmental heat stress (32,33) can raise serum S-100β greater than 0.10 $\mu g \cdot L^{-1}$ compared with intense exercise in a cool environment (control). The association reported between hyperthermia and serum S-100β values is highly variable (32), but the relationship improves when combined with dehydration and hypertonicity (33), which is expected during exercise with environmental heat stress and consistent with therapeutic principles of osmotic BBB opening (25). Although this does provide some support for animal data (12,28,29) linking heat strain and/or hypertonicity to altered BBB integrity, there is still no definitive evidence linking hyperthermia *per se* with S-100β and BBB disruption in humans, and the explanatory power of exercise with marked environmental heat stress on the appearance of S-100β deserves more attention.

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It is possible that the process of heat acclimation might afford additional insight into the effects of heat exposure on S-100β appearance in serum. In previous studies of the S-100β response to exercise and heat stress (32,33), the potential for volunteers to develop heat acclimation was carefully avoided. Heat acclimation refers to the biological adaptations conferred by repeated and prolonged exposure to exercise–heat stress (26). Among these adaptations include reduced cardiovascular and thermoregulatory strain, improved thermal comfort, acquired thermal tolerance (18), and improved exercise endurance time in the heat (15,26). It is presently unknown what other protective changes may occur, but the effects of hyperthermia on brain–blood flow (23) and neural activity (21,22) suggest the possibility that the improved exercise endurance in the heat may be rooted in an adaptive neurobiological response. Brain microvessel endothelial cells do demonstrate improved tolerance to recurrent supraphysiological heat stress (12,29), but whether this occurs in response to core body temperatures normally associated with fatigue (27) or can be gleaned from S-100β concentrations is unknown.

PURPOSE

The purpose of this study was to determine the S-100β response to moderate-intensity exercise with marked heat strain in a population vulnerable to exercise–heat exhaustion (27) before and after heat acclimation. We hypothesized that the combination of low-intensity exercise with marked heat strain would elevate S-100β levels and that heat acclimation would abate the response.

METHODS

Nine healthy male soldier volunteers (mean [range]: age = 19 yr [18–21 yr], height = 173 cm [164–180 cm], mass = 76 kg [64–84 kg], body surface area (6) = 1.87 m^2 [1.70–1.95 m^2]) participated in this study. The volunteers were physically active, took no medications, and were otherwise healthy as determined by physical examination. The study protocol was approved in advance by the Human Use Review Committee at the US Army Research Institute of Environmental Medicine and the Human Subjects Research Review Board at the US Army Medical Research and Materiel Command. Each volunteer provided written informed consent before participating. The investigators have adhered to the policies for protection of human subjects as prescribed in the Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32 CFR Part 219.

Each volunteer completed a 10-d heat acclimation protocol (15) which consisted of walking (1.56 $m \cdot s^{-1}$, 4% grade) in a hot environment (45°C, 20% relative humidity) until reaching the earliest of the three criteria: 1) 100 continuous minutes of exercise; 2) rectal temperature (T_{re}) of 39.5°C, or 3) voluntary cessation. The total exercise duration (ED) was defined as the daily endurance. Experiments were conducted in the northeastern United States (lat > 42°N) during which the high daytime air temperatures never rose above 14°C. It is therefore likely that volunteer heat acclimation state was at a natural seasonal nadir. Each morning volunteers were provided with *ad libitum* fluids and a small meal approximately 3 h before testing. An additional 250 mL of water was given 1 h before testing as a way of standardizing hydration state, that was confirmed by a serum osmolality less than 290 $mOsm \cdot kg^{-1}$. All experimental testing was conducted at the same time of the day to control for circadian fluctuations in body temperature.

Simple classic measures for successful heat acclimation (cardiovascular and thermoregulatory) were made (15,26). HR (Polar a_3; Polar Electro, Inc, Woodbury, NY) and T_{re} (Yellow Springs Instruments, Yellow Springs, OH) were measured continuously and recorded at 10-min intervals. Sweat losses were determined by the change in nude body mass pre- to postexercise. Sweat volume and mass were considered equivalent (i.e., 1 mL = 1 g) and were expressed as a rate (volume per unit time, $L \cdot h^{-1}$). The level of dehydration at the end of each trial was calculated as the change in nude body mass divided by preexercise body mass, expressed as a percentage. ED was assessed as the time taken to reach any of the three criteria for stoppage of exercise (above).

The T_{re} AUC was also calculated when T_{re} exceeded 38.5°C using a modification to the trapezium rule (11,18) where:

$$\text{AUC } (°C \cdot \text{min}) = \Sigma \text{ time interval (min)} \times 0.5 \text{ [°C above 38.5°C at the start of interval} + \text{°C above 38.5°C at end of interval]}$$

A T_{re} of 38.5°C was selected as an approximate minimum for intolerance during compensable heat stress (27) and possible threshold for S-100β appearance (31). Importantly, this value was below (−1.0°C) our laboratory safety threshold, which allowed an ample time × temperature interaction for AUC calculation. The AUC is a more accurate index of total heat stress than peak core temperature (11).

Pre- and postexercise blood samples were collected on days 1 and 10 of experimental testing. All phlebotomy was performed at room temperature (~22°C). For the preexercise blood sample, subjects reported to the laboratory after an overnight fast and sat quietly for 15 min before blood sample collection. A 5-mL whole blood sample was then taken from a forearm vein in a plain vacutainer and allowed to coagulate for 30 min before centrifugation (1000*g*) for 10 min at room temperature. The serum was aliquoted into storage cryovials and stored frozen (−20°C) until analyses. The postexercise blood sample collection was performed after the completion of exercise. Volunteers exited the chamber and sat again for 15 min before postexercise blood samples were drawn (5 mL). Analyses for S-100β were

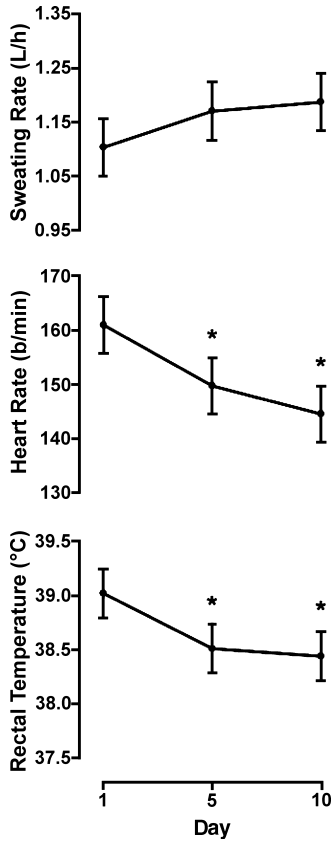

FIGURE 1—Time course of heat acclimation for sweating rate, HR, and rectal temperature. Asterisk (*) indicates day 5 and day 10 < day 1 ($P < 0.05$). Values are means ± 95% within-subjects confidence intervals (16).

performed using a commercially available enzyme-linked immunosorbant assay (Fujirebio Diagnostics AB, Göteborg, Sweden). The intra-assay coefficient of variation (CV) was 11%. Serum osmolality (freezing point depression) was measured in triplicate (CV < 1%) using a micro-osmometer (Model 210 Fiske; Norwood, MA).

STATISTICS

Physiological measurements made on days 1, 5, and 10 were compared using one-way repeated-measures ANOVA and a 95% within-subject confidence interval was calculated for each of the three time points graphed. Briefly, this procedure allows observation of a difference in the pattern of means over time. The within-subjects confidence interval displays only the interaction variance, thus shrinking the ordinate scale to improve pattern detection (16). F values requiring adjustment for sphericity were corrected using the Greenhouse–Geisser method. Blood samples taken pre- and postexercise on days 1 and 10 were analyzed using two-way ANOVA for repeated measurements. When appropriate, Tukey HSD procedure was used to identify differences among means after significant main and/or interaction effects. Pearson product moment correlation analysis was performed to examine associations among S-100β and other variables of interest.

We estimated that nine subjects would provide sufficient statistical power ($\beta = 0.20$) to detect a difference larger than the typical SD (0.04 $\mu g \cdot L^{-1}$) observed for S-100β in a normal population (1). This sample size is also sufficient to detect a difference in T_{re} larger than the typical SD (0.25°C) (3). In both cases, the magnitude of anticipated differences is consistent with previously reported observations (15,32,33). All data are reported as means ± SD except where otherwise indicated. Statistical significance was accepted at $P < 0.05$.

RESULTS

Six volunteers completed all 10 d of heat acclimation. The remaining three volunteers completed 9 ($n = 1$) or 8 d ($n = 2$) of heat acclimation, but all volunteers participated on days 1, 5, and 10. Classic physiological criteria for heat acclimation (15) were observed (Fig. 1). Physiological comparisons were made for the final values measured on days 1, 5, and 10 according to common convention (15,26). HR was significantly lower on days 5 and 10 of the HA protocol as compared with day 1, as was T_{re}. Because a blunted T_{re} elevation during exercise–heat stress is the benchmark measure of heat acclimation, AUC greater than 38.5°C was also calculated as an index of cumulative hyperthermia. Seven of nine subjects reached a T_{re} of 38.5°C or higher on day 1, while only two reached the same threshold on day 10. AUC group means were significantly lower on day 10 (1.0 ± 1.9°C·min) than day 1 (11.7 ± 10.5°C·min). Six (day 1) and eight (day 10) volunteers completed the entire 100 min of walking. As a result, ED was almost 8 min longer on day 10 (96.6 ± 10.3 min) compared with day 1 (88.8 ± 18.7 min; $P < 0.05$). Sweating rate was the same ($n = 1$) or higher ($n = 8$) on day 10 versus day 1, increasing by a mean of almost 10% ($P = 0.09$; Fig. 1). Dehydration levels achieved on days 1 (2.2 ± 0.7%), 5 (2.3 ± 0.7%), and 10 (2.6 ± 0.5%) were modest ($P > 0.05$).

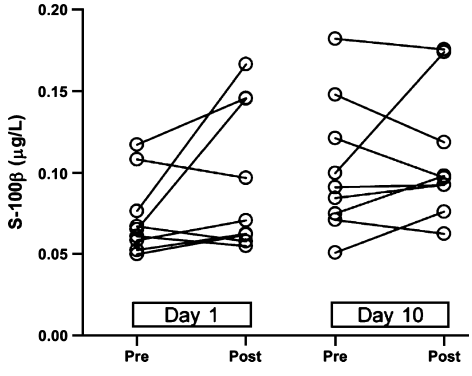

FIGURE 2—Effects of moderate exercise–heat stress and heat acclimation on serum S-100β concentrations. Individual values shown before (Pre) and after (Post) exercise on days 1 and 10 of heat acclimation.

TABLE 1. Relationship between serum S-100β and potential variables of influence.

	Correlation Coefficient	P
Peak T_{re} (°C)[a]	0.05	0.82
AUC T_{re} > 38.5°C (°C·min)[a]	0.08	0.76
Osmolality (mOsm·kg^{-1})[a]	0.13	0.48
ED (min)[a]	0.40	0.09
Δ Osmolality (mOsm·kg^{-1})[b]	0.35	0.20
Δ T_{re}[b]	0.10	0.69

All abbreviations defined in text.
[a] Pearson correlation with S-100β (μg·L^{-1}).
[b] Pearson correlation with ΔS-100β (μg·L^{-1}).

Serum S-100β concentrations pre- and postexercise on days 1 and 10 of heat acclimation are depicted in Figure 2. No differences were observed within days 1 and 10 (preexercise vs postexercise) or between days 1 and 10. The range of values for 29 of 36 samples (0.05–0.12 μg·L^{-1}) was within normal limits (1,10). The remaining seven samples were slightly above normal (0.14–0.18 μg·L^{-1}), but no significant relationships were found between serum S-100β or the change in S-100β and any variable of interest (Table 1). The mean change in S-100β (post − pre) was similar on both days 1 (0.02 ± 0.04 μg·L^{-1}) and 10 (0.01 ± 0.03 μg·L^{-1}) and well below the changes anticipated as meaningful for statistical and practical importance (0.08 μg·L^{-1}) (1). Figure 3 shows preexercise serum S-100β concentrations plotted as a function of postexercise serum S-100β concentrations. It is clear that only 3 of 18 matched pairs are biased more than 1 SD (0.03 μg·L^{-1}) in the direction of higher S-100β postexercise. Serum osmolality increased from pre- to postexercise on days 1 (preexercise: 287 ± 3 mOsm·kg^{-1} < postexercise: 292 ± 3 mOsm·kg^{-1}) and 10 (preexercise: 283 ± 5 mOsm·kg^{-1} < postexercise: 290 ± 10 mOsm·kg^{-1}; $P < 0.05$) but was otherwise similar.

DISCUSSION

This study examined the S-100β response to moderate-intensity exercise with marked heat strain and the potential for heat acclimation to alter the response. A classic heat acclimation protocol was used (15) to produce substantial thermal strain and physiological adaptation. The experiment was performed during cool seasonal conditions to ensure a common pre–heat acclimation baseline and maximize physiological adaptive responses. In addition, hydration and time of day were carefully controlled. The reduction in core temperature and HR and the increased sweating and exercise endurance time exhibited in response to 10 d of heat acclimation were similar in magnitude to observations reported by others (15). Most of this adaptation occurred by day 5 (Fig. 1) (15,26). However, acute exercise–heat stress had no effect on serum S-100β concentrations from pre- to postexercise before or after 10 d of heat acclimation.

The changes in serum S-100β from pre- to postexercise on days 1 (pre–heat acclimation) and 10 were small (~0.02 μg·L^{-1}; Fig. 2). Absolute baseline concentrations were similar to those reported by others at rest (1,24,32,33). Baseline preexercise serum S-100β concentrations on day 10 also remained unchanged by exercise (Fig. 2). Despite higher marked hyperthermia (rectal temperature) and cardiovascular (HR) strain on day 1 (Fig. 1) during exhaustive exercise–heat stress, there was no difference between S-100β concentrations postexercise on days 1 and 10. These results are consistent with many general exercise studies (19,24,30) but in contrast to recent exercise–heat stress experiments (32,33). The reason why S-100β did not increase in response to the combination of moderate-intensity exercise and severe environmental heat stress used in this study is not clear, but several possibilities exist.

The potential physiological stimuli for S-100β release among similar studies must first be considered. The level of dehydration incurred in this study was very similar to that of Watson et al. (33), but the change in osmolality was smaller, possibly the result of study differences in exercise intensity (4). The treadmill walking exercise used in this protocol resulted in postexercise S-100β change values that were slightly smaller than those observed 15 min after jogging 10 km (24). This may be because walking results in lower ground forces than running or because treadmill exercise does not place the same acceleration or deceleration forces on the brain (30) and/or muscles (9,24) as does over-ground running. Mean peak T_{re} at the end of exercise on day 1 (39.0 ± 0.5°C) was slightly higher than those reported during passive heating (38.7°C) (31) but slightly lower than other high-intensity exercise studies (39.2 to 39.5°C) (32,33). Although not reported in other S-100β studies, the T_{re} AUC greater than 38.5°C on day 1 was lower than estimates (21.3°C·min) from the first examination of exercise–heat strain and S-100β (32) but higher than estimates (1.3°C·min) from the second (33). Therefore, the hyperthermia imposed was similar to that of others evaluating the impact of exercise–heat stress *per se* on

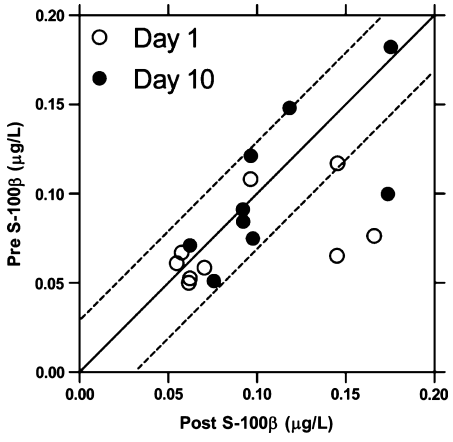

FIGURE 3—Preexercise serum S-100β concentrations plotted as a function of postexercise serum S-100β concentrations. *Solid line* represents line of identity; *dotted lines* represent 1 SD of the pooled preexercise means for days 1 and 10.

serum S-100β (32,33). It is possible that more severe (T_{re} > 41°C) hyperthermia is required (with moderate-intensity exercise or passive exposure) to elicit profound alterations in BBB integrity (12,29). It is also possible that S-100β concentrations may not accurately reflect BBB integrity during exercise–heat stress and instead have a peripheral origin associated with high exercise intensity (8,9) or the interaction of high exercise intensity coupled with hyperthermia (32,33).

The measurement of S-100β was made 15 min after the exercise cessation in this study, which is the sampling time frame used by others investigating changes in serum S-100β in response to acute exercise (5,24). Those studies making serial measurements of S-100β beginning immediately (9) or 1 h postexercise (19) show that S-100β levels can remain elevated after exercise for up to several hours. In contrast, Watson et al. (32,33) found that peak serum S-100β concentrations occurred immediately upon exercise cessation in the heat only to drop precipitously 15 min later (33). Low–molecular-weight protein extraction by the kidneys remains high even with strenuous exercise (2), and S-100β half-life appears unaffected by modest reductions in glomerular filtration rate (13). Therefore, there is little reason to suspect that S-100β clearance could have explained this drop or have likewise influenced the results reported in this study.

Although the blood sampling time frame adopted herein could be viewed as a limitation, serum S-100β has a biological half-life of at least 25 min and a decay curve that follows first-order kinetics (7,13). It is therefore reasonable that a comparison between postexercise S-100β concentrations on days 1 and 10 of heat acclimation was valid for determining potential neurobiological adaptations in the BBB as long as the short recovery period (15 min) was matched between trials. Similarly, although the convention of comparing physiological measures in response to heat acclimation at the end of exercise results in a longer ED on day 10 (average of 8 min herein), the potential for more axial brain vibrations (24), more muscle damage (9), or other phenomena (8) to increase S-100β concentrations on day 10 because of a longer ED is not supported by the data.

CONCLUSIONS

Severe hyperthermia and intense exercise can elevate serum S-100β concentrations. Because severe hyperthermia increases BBB permeability (28), it has been proposed that BBB disruption due to exercise–heat strain may be detectable using changes in serum S-100β concentrations (32,33) and that heat acclimation may protect the BBB (12,29). In this study, S-100β concentrations were not increased in response to moderate-intensity treadmill exercise with marked heat strain and no effect of heat acclimation could be interpreted for the BBB despite other classic signs of adaptation. Therefore, exhaustive exercise–heat strain (moderate intensity) does not necessarily elevate S-100β levels. Alterations in BBB integrity and conditioning of brain microvessel endothelial cells may require greater hyperthermia (12,29) than humans typically experience or can tolerate during heat acclimation programs (20). It is also possible that factors other than hyperthermia (e.g., exercise intensity) may better explain the rise in S-100β often observed with exercise.

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